

Appl. No. 10/816,579
Amdt. dated 30 August 2006
Reply to Office Action of June 05, 2006

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Amendments to the Specification:

Please amend the specification as follows:

Applicants will use both the page and line designation and a copy of the numbered paragraphs as numbered in the USPTO publication of the specification US 2005/0214168A1.

On page 13, line 14, insert ... , as shown in Figure 3, ... after the term "analyte 80 in the particles 124". Substitute paragraph [0045], in which these changes, appear, reads as follows:

[0045] The measurement of the analyte 80 in the particles 124, as shown in Figure 3, is accomplished as follows. A wire electrode 440 in the ALC 400 is set to a positive potential, and an analysis-liquid pump (ALP) 414 connected to the ACL 400 increases the pressure on the analysis liquid 800, so that a small amount of the analysis liquid 800 protrudes through a hole 430 in the ALC 400 to form a charged-volume of the analysis liquid at the hole (CVALH) 450. A pump 190 creates a vacuum in an airtight box 140 and thereby draws gas 120 and particles 124 through an induction port 126, then into a article counter 290 that counts the particles entering the APA 100, then into a charger 250 that imparts a negative charge to the particles 124, then into an airtight box 140, then past the CVALH 450, and then exhausts at least the gas 120 out of an exit port 128. As the gas 120 and particles-124 flow through the airtight box 140, at least some of the particles 124 collide with the CVALH 450 and combine with it so that the analyte 80 in the particles 124 can be measured. Electrostatic forces increase the fraction of the particles 124 that combine with the CVALH 450. The particles 124 are given a negative charge by the charger 250 so that they are drawn toward the positively charged CVALH 450 by electrostatic forces. When the CVALH 450 is negatively charged the particles 124 are given a positive charge by the charger 250 so they are drawn to the CVALH 450. A shield electrode [[490]] 480 around the hole 430 is given a voltage opposite to that of the CVALH 450, and of much smaller magnitude than the voltage applied to the CVALH 450, so that particles are not attracted to this region around the hole 430, but to the CVALH 450. More than one particle 124 may combine with the CVALH 450. If one or more of the particle(s) 124 that mix with the CVALH 450 contain some analyte 80, the

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fluorescence of the CVALH 450 begins to change in accordance with the amount of the analyte 80. After a time sufficient for the analyte 80 to react with the analysis liquid 800 and to generate fluorescence, the fluorescence of the CVALH 450 is measured as explained below. The amount of the analyte 80 in the particles that collided with the CVALH 450 is determined from this measured fluorescence. Then the ALP 414 increases the pressure on the analysis liquid 800 in the ALC 400 so that a CVALH 450 is ejected and falls into a receptacle 470 at the bottom of the airtight box 140.

On page 14, line 11, please change the term "shield electrode 490" to ...shield electrode 480... as shown in the substitute paragraph [0045] above.

On page 14, line 17 at end of the line, change the term "analysis liquid 80" to read ...analysis liquid 800...as shown in the substitute paragraph [0045] above.

On page 17, line 2, change the term "particle counter 190" to ... particle counter 290 ... as shown in substitute paragraph [0050] that follows.

[0050] A particle counter ~~[[190]]~~ 290 measures the concentrations of particles 124 in different size ranges drawn into the APA 100 so that when the APA 100 is calibrated the mass of the particles 124 that combine with the CVALH 450 can be estimated from a lookup table, so that the concentration of the analyte 80 in the particles can be determined from the measurements of the amount of analyte 80 in the particles 124 and the estimated mass of the particles 124 that combined.

On page 17, line 9, please change the term "shield electrode 442" to read ...shield electrode 480... as shown in the substitute amended paragraph [0051] that follows.

[0051] The ALC 400 with the hole 430, the ALP 414, and the electrode 440 with the shield electrode ~~[[442]]~~ 480 together comprise the analysis-liquid handling subsystem (ALHS) 456. In one exemplar, the ALC 400, near the hole 430, is circular as in a typical capillary tube.

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On page 20, line 20, change "Stem-2 822" to ...Stem-2 **824**... Please note that in the printed version, [0057], the Stem-#s through out the paragraph and the printed publication are not separated from the numeral identifying the part as shown in the substitute paragraph [0057] that follows.

[0057] **FIG. 3** illustrates schematically one embodiment of the reaction that takes place in the CVALH **450** when analyte **80** is present in the particle **124** that combined with the CVALH **450**. **FIG. 3** shows how the fluorescence of the analysis liquid **800** changes, so that the fluorescence of the CVALH **450** varies with the amount of analyte **80** in the particles **124** that combined with the CVALH **450**. The example shown in **FIG. 3** is the same as that shown in **FIG. 6A** of an article by R.L. Nutiu and Y. F. Li, "Structure-switching signaling aptamers," Journal of the American Chemical Society, 125, 4771-4778 (2003), (herein incorporated by reference, especially **FIG. 6A**). In **FIG. 3**, the structure-switching signaling aptamer **820** is comprised of: (i) an aptamer (MAP) **822** chosen because it binds selectively to the analyte **80**, i.e., it acts as a sensor molecule, (ii) a DNA oligonucleotide, Stem-2_824, which is covalently linked to the MAP **822**; (iii) a DNA oligonucleotide, Stem-1 **826** that is covalently linked to Stem-2 [~~822~~] **824**; (iv) a fluorophore (F) **832**; (v) DNA oligonucleotide (FDNA) **834** that is linked to the fluorophore **832**; (vi) a quencher (Q) **828**; and (vii) a DNA oligonucleotide (QDNA) **830** that is linked to the quencher **828**.

[0058] The FDNA **834** forms the DNA duplex with Stem-1_826. The QDNA **830** forms the DNA duplex with Stem-2_824. In this structure-switching signaling aptamer **820**, the fluorophore **832** and the quencher **828** are held near each other and the quencher **828** quenches the fluorescence of the fluorophor **832**, so that the fluorophor **832**, fluoresces very weakly if at all. When the analyte **80** is present, the MAP **822** of the structure switching signaling aptamer **820** binds to the analyte **80** as illustrated in **FIG. 3**, and thereby releases the QDNA **830** so that the fluorophore **832** is no longer quenched, and can fluoresce brightly. In another exemplar, for cases where the analyte **80** is an oligonucleotide, the approach illustrated in **FIG. 3** is used, but for these analytes **80** the aptamer (MAP) **822** is replaced by an oligonucleotide that is complementary to the analyte **80**. In another exemplar, the analysis liquid contains a molecular aptamer beacon as described by J. W. J. Li, X. H. Fang and W. H. Tan in, "Molecular Aptamer Beacons

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for Real-time Protein Recognition," Biochemical and Biophysical Research Communications, 292 (1), 31-40 (2002), incorporated herein by reference. A description of methods for generating and using aptamers and molecular beacon aptamers is in U.S. Pat. No. 6,531,286 B2, "Homogeneous detection of a target through nucleic acid ligand-ligand beacon interactions," by S. Jayasena and L. Gold. In other exemplars, the sensor molecule may be, an antibody or a phage-displayed epitope or another protein, or it may be a nucleic acid selected to bind to a DNA or RNA sequence from the analyte organism.

On page 23, line 20, please change As shown in the substitute paragraph [0061] that follows.

[0061] Although only the measurement of the fluorescence intensity is described here in detail, other fluorescence properties such as the fluorescence polarization, the fluorescence spectrum, and the fluorescence lifetime can also be used in some embodiments of the APA 100, and methods for measuring these properties are well enough known, that more does not need to be stated here. Also, methods for measuring other optical properties such as light scattering properties related to, for example, the measured polarization, spectral intensity, and angular-dependent intensity, have been described by other researchers. Although only the measurement of one analyte is described here in detail, the extension to the measurement of multiple analytes using multiple recognition molecules and multiple fluorophors that have different emission spectra is similar enough to what has been done in other analyses. Also, multiple recognition molecules can be used to detect multiple sites on the same analyte, as is well known. The use of electrostatic forces to deflect charged particles or droplets into different containers, depending upon some measured property of the droplet, e.g., using chargeable deflection plates, is known and has been used with flow cytometry; the sorting of charged droplets of the analysis liquid ~~[[460]]~~ 450 using electrostatic deflection after they are ejected can also be used with this invention.